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Within Season Hematological Changes in College Athletes with Sickle Cell Trait

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WITHIN SEASON HEMATOLOGICAL CHANGES IN COLLEGE ATHLETES WITH SICKLE CELL TRAIT

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ABSTRACT

PURPOSE: To explore the hematological differences in African-American athletes with sickle cell trait (SCT) and healthy controls (CON) before preseason camp and the changes that occur after a full season. **METHODS**: Sixteen (n=8 SCT; n=8 position matched, CON) NCAA Division 1 athletes (12 football, 2 each women's soccer and volleyball) had blood drawn before preseason camp, after and at the end of the season, and analyzed for Hb electrophoresis (Hb-A, Hb-A2, Hb-S, and Hb-F), complete blood count with differential, chemistry panel 26, and prothrombin time, activity and international normalized ratio (INR). **RESULTS**: Baseline total Hb was similar between SCT and CON (mean±SD; 14.2±1.3 vs. 14.1±0.9g/dL, resp; P=0.87), and as expected, Hb-A was lower and Hb-A2 and Hb-S were higher in SCT than CON (P<0.001 for all). Baseline neutrophils were higher (4.43±1.29 vs. 3.28±0.93cellsx10^3/mL, P<0.005) and lymphocytes tended to be lower (1.56±0.45 vs. 1.99±0.39cellsx10^3/mL; P=0.09) amongst SCT compared to CON, respectively. Baseline amylase (99.4±25.4 vs. 72.9±18.8u/L; P=0.03), uric acid (6.8±1.7 vs. 5.5±0.8mg/dL; P=0.08), and creatinine (1.2±0.2 vs. 1.0±0.2mg/dL; P=0.07) were higher in SCT compared to CON. All Hb measures in SCT were similar after camp compared to baseline (P>0.33 for all). Baseline and position adjusted change in neutrophils were similar between SCT and CON (mean, 95%CI; -0.09, -2.06 to 1.89 vs. 0.36, -1.61 to 2.34cellsx10^3/mL, resp; P=0.77) from preseason to post-camp. Similarly, the adjusted change in lymphocytes was not different between SCT and CON (0.53, 0.01 to 1.05 vs. 0.15, -0.37 to 0.67, resp; P=0.40), however, total lymphocyte counts increased in SCT over the time from preseason to post-camp (P<0.05). Creatinine responses differed between SCT and CON (-0.05, -0.12 to 0.02 vs. 0.05, -0.03 to 0.12; P=0.05) and potassium decreased a greater extent in SCT (-0.44, -0.6 to -0.3 vs. -0.2, -0.3 to -0.1; P=0.05) after camp. Complete blood counts in SCT and CON were

similar across all measures (P>0.07 for all) when comparing preseason to postseason. The adjusted change in sodium (-0.44, -1.22 to 0.35 vs. 1.13, 0.38 to 1.87; P<0.006) and GGT (0.95, -2.73 to 4.62 vs. 5.32, 1.77 to 8.88; P<0.02) showed significant difference between SCT and CON, after season compared to baseline. **CONCLUSION**: Despite major hematological differences due to SCT, very few changes occurred during the exhaustive, preseason camp and regular season at sea level.

CHAPTER 1. LITERATURE REVIEW

Introduction

The research on sickle cell disease (SCD) in sports has made major progress over the years, but still results remain inconclusive about individual's ability to perform exercise and/or sport, and what possible hematological changes occur because of exercise and sport. Sickle cell disease is the most common inherited blood disorder amongst people, a mutation in the hemoglobin-beta gene found on chromosome 11, causing hemoglobin (Hb) to become abnormal. Normal hemoglobin takes the shape of a biconcave disc, while sickle hemoglobin is shaped much like a crescent, or sickle. Sickle cell trait (SCT) is passed through generations from carriers, prominently of the African-American, Middle Eastern, and Mediterranean descents. Sickle cell anemia (SCA) consist of sickle hemoglobin (Hb-S) replacing more than 50% of hemoglobin A (Hb-A). On the other hand, is sickle cell trait (SCT) which is known to be milder, than anemia, that has less than 50% of Hb-A in the blood. Both sickle hemoglobin and Hb-A are both found in the blood of SCA and SCT individuals, but the former has more than a majority of Hb-S. The hemoglobin protein in red blood cells (RBC) is the carrier of oxygen through the blood. In individuals with SCT, low oxygen levels induce erythrocytes sickling. The sickling comes from the mutated beta chains contracting and piling together, causing the shape. Once a cell is sickled, it becomes rigid and clumps together, causing the cell to become more fragile. Being more fragile increases the erythrocytes probability of being broken more easily, which decreases the cell life span by approximately 90%, lasting 10-12 days in the blood compared to the 120 days of normal blood cells, in SCA individuals. The fragile and sticky sickle cells alter deformability, the ability to conform shape for efficient flow through constricted or dilated vessels, giving rise to the probability of blocked blood flow, ultimately, causing hypoxia, and

inhibiting oxygen availability to tissues and organs. The inability of a RBC to change shape to pass through circulation leads to blood flow resistance, ultimately preventing oxygenated blood passing through vessels and bringing oxygen and nutrients to tissues. [1] Rhabdomyolysis, splenic infarction, and medullary sickling are three concerns associated with sickle cell, in sport and/or exercise. [2] Other factors that are known to increase sickling include, being hypohydrated and dehydration, infection, alcohol consumption, high altitudes, extreme environmental or body temperatures, acidosis, and strenuous exercise, amongst others. Crises is a period of extensive cellular sickling, they can occur from exposure or participation in all the scenarios previously stated.

Exercise and Sickle Cell

Whether an individual has SCA or SCT, the individuals, coaches and staff need to take the necessary precautions of being alert and learning the signs and symptoms. Some signs and symptoms include muscle pains, starting in the back and lower body, weakness, undue fatigue, breathlessness, muscle cramps, altered mentation. [3-7] Other features of an athlete could include the individual slumping to the ground, but still have the ability to speak. [3] Previous research has further looked at military, athletes, and individuals during various types of exercise. Many of the exercise protocols range from aerobic exercises such as the, 6-minute walk test, cycling ergometers, to high intensity sprints, all of which are attempting to emulate the same efforts performed during competitions or trainings. [1, 8] How well an individual with SCT is able to perform different tasks, associated with exercise, in comparison to an individual without SCT, or a control (CON), has shown various different results. In a cycle ergometer protocol, many of the results, VO_{2max} (mean±SEM; SCT: 38.6±2.6, CON: 43.3±2.5mL/kg^{-1} min^{-1}), maximal heart rate (HR_{max}) (mean±SEM; SCT: 177±6, CON; 177±10 beats/min^{-1}), maximal power output

(mean±SEM; SCT = 283±12, CON: 286±8W), plasma viscosity (mean±SEM at rest; SCT: 1.69±.04, CON: 1.72±.02mPa/s^{-1}), hematocrit (Hct) (mean±SEM at rest; SCT: 45.75±.69, CON: 46.21±.44%) and lactate concentration (mean±SEM at rest; SCT: 1.51±.13 , CON: 1.58±.13mM) were said to be insignificant or displayed no difference. [8] As stated previously, after maximal effort cycling RBC's continued to have an increased risk of causing blockage, due to an inability to change shape to move through vessels. [8] In addition to maximal effort exercise, extended periods of strenuous exercise causes a greater increase in blood viscosity, higher RBC lipid oxidative stress and higher leukocyte and platelet activation in SCT carriers. [9] In a chronic study examining carrier status association with fitness levels, researchers described how SCT carriers were at an increased risk of kidney disease, and thrombosis, amongst other hematological issues. [10] The increased risk also prompted the authors to consider the carriers' risk for metabolic syndrome. Over a 25-year period, baseline and longitudinal changes of the 136 SCT carriers showed no significance, for metabolic syndrome or in fitness parameters. Liem et al. stated, there was not a difference between SCT status and the incidence of diabetes (n=136 SCT, n=1859 CON; means; SCT: 0.0, CON: 0.8%; P=.29) or metabolic syndrome (means; SCT: 2.2, CON: 2.0%; P=.93), among the African American population. [10] In addition to the research investigating acute responses to exercise, post-exercise training research had to discern crises occurring within hours after exercise. After looking over the blood viscosity, plasma viscosity, Hct, deformability/ RBC rigidity, and other hemorheological parameters, the authors found higher blood viscosity, at rest (mean±SEM; SCT: 5.01±.29, CON: 4.78±.14mPa·s) and 24 hours post exercise (mean±SEM; SCT: 4.55±.16, CON: 4.39±.12mPa·s) in SCT individuals compared to the healthy trained participants. Hct was found to be lower in the SCT group, and RBC rigidity was higher across all three time points, rest, end of maximal exercise and 24h post-

exercise. [11] Though some studies findings seem to show no significance in the comparison of SCT and none SCT individuals exercising, the number of incidents and crises occurring due to SCT complications still gives reason to continue researching effects and differences of acute and chronic exercise and SCT.

Sports and Sickle Cell

Similar to exercise, sports can vary in the amount of exertion needed in order to perform the given task. Over many years, numerous incidents, crises, or death have occurred in sport due to SCT. Some of which, happened in the beginning moments of maximal or submaximal efforts, or after extended periods of strenuous exertion. [3, 4, 12, 13] For many years, there was controversy on whether college athletes should be tested for SCT but following multiple deaths in the first decade of the year 2000, rules were changed to require testing, annually, for SCT in all athletes whose status was unknown. [6] The first football related collapse occurred on an athlete's first day of practice in two consecutive years. The athlete, Polie Poitier, collapsed after running near 700 meters of sprints. After being pronounced dead the following day, the cause was said to be acute exertional rhabdomyolysis associated with SCT. Unfortunately, being ignorant to signs and symptoms related to a SCT crises, is no longer valid reasoning for overexerting an athlete during training. [14] Since then, multiple other occasions in which young athletes passed away due to complications associated with SCT occurred. Ereck Plancher, Devaughn Darling, Aaron O'Neal, and most notably Dale Lloyd, were some of the few who tragically suffered from SCT related deaths while performing their sport. Dale Lloyd's death was responsible for the mandated screening of SCT in all athletes. [14] The National Collegiate Athletic Association (NCAA) in April 2010 started the policy to provide necessary education on SCT. The new policy demanded all incoming Division I student-athletes be tested for SCT. [6]

With the exception of Plancher, Darling, O'Neal, and Lloyd all collapsed during off-season workouts, while Plancher's collapse was post workout, during the off-season. Off-season is the time when athletes have been resting from the previous season, followed by workouts and trainings in order to help prepare, and better, them for the upcoming season. Off-season training can be very tough, depending on the amount of exercise and conditioning an athlete completed on their own before the required off-season team workouts. The risk of exertional death in all football players with SCT was 37-fold higher, than in athletes without SCT. [12] Hypoxemia, acidosis, hyperthermia, and red-cell dehydration are all forces evoked by sustained exercise in athletes with sickle cell. [3] As Eichner states, there is a common denominator for sickling collapse in athletes with SCT, it is maximal exertion sustained for more than a couple minutes. [3] Though sport and exercise are similar, the efforts and exertions given, or expected, during competition can be very different. Could research be studying during the wrong seasons, or periods? Is there a work to rest ratio that must be implemented? Finding the underlying mechanisms that lead to incidents, crises, or deaths due to SCT related complications are continually taking steps to improve, but further research is still needed.

Environmental Effects

Though maximal exertion seems to be the leading cause to athletes with SCT collapsing, other factors, too, have just as important of a role in causing exertional sickling. The influence of environmental factors has not been researched often, but the few that have, found similar results of increased sickling due to various weather conditions, in studies wanting to find if there was an association of hospital admissions in relation to the environment in individuals with SCD. Environmental influences relate to temperatures, humidity, rainfall, wind speed, etc., all of which have been researched for extended periods of time. As suspected in a study by Dessap (2014), as

the temperatures decreased, the risk of SCD individuals going to the hospital increased. [15] The cold temperatures are not likely to cause sickling, but rather a constriction reflex on blood vessels, due to the temperature of the skin lowering, which most likely resulted in an obstruction in the vessels due to the sickled RBC's inability to conform their shape. Much like the cooler temperatures from the study just mentioned, rainfall, and wind speed emerged as important environmental influences to increase SCD related hospitalizations. Higher wind speeds and rainfall both can cause a person to feel much colder, which has been suggested as a cause of vaso-occlusive pain, or lower thermal stress [16]

Another very important environmental influence that has the potential to cause sickling of RBC's and possibly lead to other organ damage is altitude. Exercise in high altitude environments (above 1100m) has been shown to be correlated to an increased risk for splenic infarction in SCT carriers. [9] Altitude changes have a massive effect on partial pressures of an individual's lungs and breathing, whether with sickle cell or having normal hemoglobin. From sea level (<500m) to extreme altitudes (>5500m) the partial pressures working to exchange the gases, oxygen and carbon dioxide, become affected, by the reduced partial pressures of oxygen in the higher altitude. So, as the altitude increases, maximal oxygen uptake begins to decline. [17] A former professional football player, suffered life threatening complications from SCT while in Denver, Colorado (5280 feet). Ryan Clark was withheld from participating in the game due to sudden pains, which came from the blocked vessels induced by exertional sickling. The sickled cells prevented the oxygenated blood cells from reaching his spleen, ultimately causing splenic infarction which led to the removal of his spleen and gall bladder. [14] Another research study showed that extreme altitude also has a detrimental effect on aerobic exercise. During this 34.1km race, in altitudes of 3800+ meters, athletes with SCT showed significant performance

decrements, in comparison to their matched non-SCT runners (means±SD; SCT=52.0±11.1mins vs CON=45.2±9.7mins; P<0.01). [18] Knowing all the different environmental factors that have a potential effect on sickling of RBC's, can help individuals understand the necessary precautions that need to be taken when preparing, or performing in the various conditions.

Stressors

In life and exercise, other factors can result in stress in individuals with SCT. Some of those factors can be, being hypohydrated, in poor physical condition, as well as inflammation or oxidative stress, which both have immediate responses to exercise or activity. Staying hydrated is a vital part of living, and the inability to maintain fluid balance, prior or during exercise, can cause stress on an individual with SCT. Fluids help transport nutrients and remove waste, but they can also assist with body temperature regulation. The previous section demonstrated how temperature changes can affect circulation, and several studies examined the role hydration has in an individual with SCT, euhydrated and hypohydrated, in comparison to CON participants. Tripette et al., looked at SCT and non-SCT, in both a dehydrated state and hydrated state and examined hemorheological responses after submaximal exercise. In response to exercise, the researchers found the plasma viscosity (means±SD; Dehyd: 1.58±.07, 1.68±.18 mPa/s; Hyd: 1.53±.07, 1.67±.20 mPa/s) increased in SCT athletes, no matter hydration status, in comparison to their baseline values. [19] The participants blood viscosity, in both articles, found similar results, with SCT athletes having greater blood viscosity in comparison to CON, and the dehydrated group of SCT athletes increasing their blood viscosity during recovery or post competition. [19, 20] As blood flow becomes slower following exercise, and in comparison, to non-SCT at rest, surprisingly RBC rigidity decreased following exercise, without regard to hydration. The change, between being hydrated or not, was observed during the 2-hour recovery

period, when RBC rigidity greatly increased past initial values, in the dehydrated SCT athletes, and the hydrated SCT athletes steadily decreased their RBC rigidity. [19] Despite not knowing whether the individuals are euhydrated prior to exercise, making sure SCT individuals have water to consume during exercise has shown to benefit blood flow, during and following exercise.

Some of the most harmful effects of sickling include, endothelial dysfunction, inflammation, and vaso-occlusion, which all arise from increased oxidative stress. Oxidative stress is the imbalance in the making of antioxidants and oxidants. [21] Various articles have found that chronic exercise has improved the balance of oxidants and antioxidants, decreasing the oxidative stress. In one study that determined whether regular training improves oxidative stress, in untrained and trained, SCT and non-SCT, the participants performed incremental maximal exercise on a cycle ergometer. Aside from the performance results, SCT and CON show similar maximal aerobic power, the trained SCT had higher platelet counts than untrained SCT and CON. Antioxidants such as superoxide dismutase (SOD) (means±SD; 3.4±1.4, 8.7±7.7μmol·ml^{-1}·min^{-1}), and nitric oxide metabolites (NO_x) (means±SD; 19.8±3.1, 28.8±11.4μmol.1^{-1}) all showed significant increases following exercise, compared to baseline, in trained individuals. In addition, glutathione peroxidase (GPX), another antioxidant, increased compared to baseline, but without regard to Hb or training status. [21] In a different study, the testing protocol called for mild to moderate endurance exercise for 20 mins in SCA individuals and non-SCT, CON. Faes et al. determined the effects of the exercise protocol on oxidative stress, adhesion and NO bioavailability. Though expected, in comparison to the more benign SCT study, the authors found differences in performance as well as with oxidative stress, negatively affecting SCA individuals. Following exercise, SCA NO_x was significantly lower, platelet count, white blood cells,

and GPX were all significantly higher, all compared to baseline. [22] Though most of the changes post exercise were similar to those in the CON group, the fact that the SCA individual's baselines values varied significantly in nitrotyrosine (means±SD; CON: 57.9±24.2, SCT: 207.9±60.4nmol/l), GPX (means±SD; CON: 8.4±4.3, SCT: 11.2±7.7nmol/l/min), ferric reducing antioxidant power (FRAP) (means±SD; CON: 1076±207, SCT: 1343±322µmol/l), and NO_x (means±SD; CON: 22±10, SCT: 14.1±8.3µmol/l), showed nonbeneficial to preventing oxidative stress. [22]

To know that a well hydrated SCT athlete can reduce the potential of sickling RBC's from blocking vessels, by augmenting vasodilation, shows there are still ways to improve the performance and health of these individuals. In addition, staying active can also help with maintaining the balance of antioxidants and oxidants in response to exercise. Though some results show improvements in the ability of blood to flow, or decrease sickling, and damage to organs, other factors such as, pre-exercise hydration, illness, climate, exertion, and situational individual responses may still play a role in causing sickling or incidence.

Purpose

We explored the biological, physiological, and psychological effects of division I-A collegiate level training on individuals with SCT. In order to examine these relationships, we recruited college level athletes with SCT and tested them for hematological changes that occurred in a season, from rest (preseason), throughout their season (camp), and at the conclusion of their seasons (postseason) in their respective sports. We hypothesized that, the athletes would have minimal differences in ability to perform their necessary task, but we expected some hemorheological changes would be more noticeable in the SCT athletes, following postseason.

CHAPTER 2. METHODS

Participants

We recruited sixteen (n=8 SCT; n=8 CON) NCAA Division 1 athletes (12 football, 2 each from women's soccer and volleyball) with and without SCT. The SCT athletes were position matched with CON, in attempt to match phenotypes and physical training. Athletes were recruited by word of mouth and contacted by their athletic trainers. All the athletes were offered the informed consent, provided time to review the documents, and signed the content prior to baseline testing and their screening data.

Inclusion/Exclusion Criteria

Only active athletes in their respective sports with confirmed SCT were included in the study. SCT was determined by their health history provided by the medical staff or athletic trainers and confirmed by their baseline laboratory screening. Conversely, the position-matched athletes were confirmed to be without SCT, by the staff, and served as the control (CON) group. Participants could be excluded if principle investigators deemed necessary due to other medical, psychiatric, or behavioral factors that would interfere with study participation or ability to follow protocol.

Study Design

Blood chemistry data was collected before (Pre-season), during (Camp), and after (Post-season) the season, in all sixteen athletes. Any data we collected during Camp was due to the occurrence of an "event" with one of the sickle cell athletes. Events were deemed as such by the athletic training staff of any incident that resulted in dizziness, extreme fatigue, lethargy, cramping, or any other related symptomology due to SCT. Data on the position-matched

participant was also collected if an event occurred. In addition, data was collected before, during, and after scheduled practices during pre-season camp. Practice data included body weight, urine specific gravity and electrolytes, sweat rates, skin and core temperatures, heart rate and heart rate variability (HRV), and breathing rate, but we limited the analysis to only hematological data for this manuscript.

Practices, and any other sport related activities done by the athletes, were conducted by their respective coaches. Investigators had no influence on the physical activity training, or sport-specific physical performance testing of the athletes. Environmental conditions were also collected to determine possible correlations between weather and athlete's ability to perform. Practices consisted of team warmups and stretching, installation walk-thru, skill drills with their position groups, individual, team, and offense vs. defense. Practices were conducted indoors and outdoors.

Visits/Assessments

Pre-season data served as baseline measurements, while follow-up measurements, camp, post-season or events, examined chronic changes associated with practice and competitions throughout the season. Blood samples from each participant consisted of 10 mL of plasma (EDTA) and 10 mL of serum, at each time point and examined at Pennington Biomedical Research Center Clinical Laboratory for changes in hemoglobin, complete blood cell counts, white blood cell counts, amongst other hematological properties associated with SCT. Additional blood samples were collected when medical events occurred and were analyzed for similar parameters listed above and for performance enhancing supplements. These outcomes allowed us to examine additional unknown interactions that may occur in SCT athletes.

Statistical Analysis

All statistics were performed using JMP version 13.0 statistical software (SAS Institute Inc., Cary, NC). Data analysis followed CONSORT recommendations using General Linear Models and repeated measures analysis of variance (RM-ANOVA), co-varied as needed depending on normality distributions at baseline. Paired (by position-matched group) and independent t-tests were used to compare group differences at each time-point (Pre-season, Post-camp, and Post-season). No adjustments for multiple comparisons were used given the pilot nature of this study. In addition, two-way RM-ANOVAs (SCT x time-point) were used to determine differences in responses in all hemorheological variables. Student's t tests were used to determine significant effects when appropriate. Baseline adjusted change scores (post-camp – pre-season; post-season – pre-season) were also presented due to the potential effects of the midpoint testing on the RM-ANOVA modeling. Baseline data were reported as mean±SD and baseline adjusted change scores were presented as mean±95%CI. Significant differences were declared at $P<0.05$.

CHAPTER 3. RESULTS

Table 1. Baseline Participant Characteristics

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
n	12	6	6	-	-	
Age (y)	20.3±1.6	19.6±1.9	20.8±1.2	0.22	0.16	0.42
Weight (kg)	115.7±24.7	117.0±27.9	114.4±23.7	0.86	0.48	0.12
Height (cm)	189.9±5.1	187.1±5.2	192.6±3.4	0.06	0.07	0.81
Total Hb	14.6±0.8%	14.8±0.8%	14.4±0.8%	0.47	**0.007**	0.12
Hb-A	76.0±20.1%	56.8±0.9%	95.2±0.4%	**<0.0001**	**<0.0001**	0.47
Hb-A2	3.13±0.4%	3.5±0.2%	2.8±0.2%	**0.0001**	**0.002**	0.69
Hb-S	19.2±20.1%	38.5±0.9%	0.0%	**<0.0001**	**<0.0001**	>0.99
Hb-F	0.4±0.3%	0.4±.0.3%	0.5±0.3%	0.26	0.40	0.21
Hb-C	0.0	0.0	0.0	-	-	

Values are mean±SD. Between analysis for SCT vs. NORM; Within participants analysis accounts for individuals variability with matched pairs. Bold values are considered significant. Pair within is the Wald p-value for the random variability.

Table 2. Baseline Complete Blood Counts

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
Red Blood Cells (RBC) ($x10^6$)	5.0±0.4	5.2±0.3	4.9±0.4	0.09	0.20	0.29
Hematocrit (Hct)	42.4±2.0%	43±2.5%	41.8±1.5%	0.36	0.20	0.30
Mean Cell Vol. (MCV) (fL)	84.5±6.4	82.3±3.4	86.8±8.2	0.24	0.14	0.34
MCH (pg)	29.1±2.8	28.3±1.2	30.0±3.7	0.31	0.25	0.52
MCHC (g/dL)	34.4±0.9	34.4±0.5	34.4±1.3	0.98	0.98	0.54
RDW	13.8±1.1%	14.3±1.0%	13.3±1.0%	0.14	0.09	0.38
Platelet Count ($x10^3$)	232.9±54.2	224.0±67.1	241.8±42.0	0.59	0.62	0.86
White Blood Cell (WBC) ($x10^3$)	6.4±1.2	6.7±1.4	6.1±1.0	0.41	0.13	0.17
Neutrophils	62.2±11%	68.1±9.8%	56.4±9.3%	0.06	**0.04**	0.44
Lymphocytes	28.2±9.3%	23.7±7.7%	32.8±9.1%	0.09	**0.04**	0.32
Monocytes	7.5±1.9%	6.7±2.5%	8.2±0.8%	0.17	0.15	0.61
Eosinophils	1.5±1.0%	1.0±0.7%	2.0±1.1%	0.09	0.19	0.31
Basophils	0.5±0.2%	0.5±0.2%	0.5±0.2%	0.90	0.89	0.73
Neutrophil # ($x10^3$)	4.0±1.3	4.6±1.4	3.5±1.0	0.13	**0.005**	0.14

(Table Cont'd.)

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
Lymphocytes # ($x10^3$)	1.7±0.5	1.5±0.5	2.0±0.5	0.15	0.21	0.76
Monocytes # ($x10^3$)	0.5±0.1	0.4±0.2	0.5±0.1	0.51	0.60	0.36
Eosinophils # ($x10^3$)	0.1±0.1	0.1±0.1	0.1±0.1	0.19	0.29	0.41
Basophils # ($x10^3$)	.02±.04	.02±.04	.02±.04	1.00	-	**0.03**
Reticulocytes	1.4±0.5%	1.3±0.5%	1.5±0.6%	0.62	0.56	0.50

Values are mean±SD. Between analysis for SCT vs. NORM; Within participants analysis accounts for individual's variability with matched pairs. Bold values are considered significant. Pair within is the Wald p-value for the random variability.

Table 3. Baseline Blood Chemistries

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
Glucose (mg/dL)	87.5±12.6	87.4±11.2	87.7±15	0.97	0.98	0.78
Sodium (mmol/dL)	136.8±1.3	137.0±1.7	136.6±1.0	0.61	0.69	0.30
Chloride (mmol/dL)	103.7±1.1	103.5±0.8	104.0±1.4	0.44	0.48	0.83
Potassium (mmol/dL)	4.5±0.3	4.6±0.4	4.4±0.3	0.56	0.64	0.40
Carbon Dioxide (mmol/dL)	25.2±1.2	25.3±1.7	25.2±0.5	0.82	0.83	0.96
Uric Acid (mg/dL)	6.6±1.3	7.3±1.4	5.9±0.5	**0.04**	**0.05**	0.61
Calcium (mg/dL)	9.5±0.3	9.5±0.3	9.5±0.2	0.92	0.91	0.64
CPK (IU/L)	356.0±131.2	386.8±164.6	325.3±92.3	0.44	0.51	0.54
LDH (IU/L)	225.0±68.9	226.2±93.8	223.8±40.4	0.96	0.97	0.29
AST (IU/L)	32.5±7.7	32.7±9.5	32.3±6.2	0.94	0.95	0.44
ALT (IU/L)	26.8±11.8	26.7±10.5	27±14	0.96	0.96	0.69
ALK Phosphatase (IU/L)	82±26.9	85.6±28.9	78.3±26.8	0.66	0.72	0.42
GGT (IU/L)	18.3±14.7	21.3±14.8	15.3±15.2	0.50	0.53	0.91
Amylase (U/L)	80.6±26	98.2±26.9	63.0±4.5	**0.01**	**0.02**	0.70
Iron (µg/dL)	101.7±30.7	96.7±16.5	106.7±41.7	0.60	0.47	0.31
Cholesterol (mg/dL)	171.2±20.8	177.5±26.7	164.8±12.1	0.31	0.34	>0.99

(Table Cont'd.)

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
Triglycerides (mg/dL)	138.4±70.0	133.5±67.6	143.3±78.4	0.82	0.84	0.68
HDL Cholesterol (mg/dL)	56.8±16.9	56.1±19.1	57.5±16.3	0.89	0.90	0.80

Values are mean±SD. Between analysis for SCT vs. NORM; Within participants analysis accounts for individual's variability with matched pairs. Bold values are considered significant. Pair within is the Wald p-value for the random variability. *insufficient blood samples from multiple position-matched participants.

Table 4. Camp Hemoglobin Levels

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
Total Hb	14.0±0.7	14.1±0.7	13.9±0.7	0.67	0.18	0.13
Hb-A	75.9±20.2	56.6±0.3	95.2±0.3	**<0.0001**	**<0.0001**	0.35
Hb-A2	3.1±0.4	3.5±0.1	2.8±0.1	**0.0002**	**0.004**	0.54
Hb-S	19.3±20.2	38.7±0.3	0±0.3	**<0.0001**	**<0.0001**	>0.99
Hb-F	0.4±0.3	0.4±0.1	0.5±0.1	0.27	0.41	0.20
Hb-C	0	0	0	-	-	-

Values are mean±SD. Between analysis for SCT vs. NORM; Within participants analysis accounts for individual's variability with matched pairs. Bold values are considered significant. Pair within is the Wald p-value for the random variability.

Baseline Data

Anthropometric data at baseline are shown in Table 1. The table shows there was no significant differences between or within our SCT and CON groups, confirming our position matches to being similar. In addition to the anthropometric data, baseline hemoglobin data showed many significant differences, also in table 1. Total Hb (within: P=0.007), Hb-A (between: P<0.0001; within: P<0.0001), Hb-A2 (between: P=0.0001; within: P=0.002), and Hb-S (between: P<0.0001; within: P<0.0001) were all significant, with SCT being higher, lower, higher and higher, resp. in comparison to the CON. SCT participant's basal values for neutrophils (68.1±9.8% vs 56.4±9.3%; within P=0.04) and neutrophil number (4.6±1.4x10^3 vs 3.5±1.0x10^3; within P=0.005) were elevated, and lymphocytes (23.7±7.7% vs 32.8±9.1%; within P=0.04) were lower than their CON matches, as shown in Table 2. From Table 3, uric

acid (SCT= 7.3±1.4mg/DL vs CON=5.9±0.5mg/DL; between P=0.04, within P=0.05) and amylase (SCT=98.2±26.9u/L vs CON=63.0±4.5u/L; between P=0.01, within P=0.02) were the only two measures to show significance, as individuals and when looked at as the same person.

Follow-up Data by Time Point

Camp

Table 5. Camp Complete Blood Counts

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
Red Blood Cells (RBC) ($x10^6$)	4.9±0.4	5.0±0.2	4.7±0.5	0.17	0.22	0.75
Hematocrit (Hct)	40.8±1.9%	41.1±2.3%	40.7±1.6%	0.71	0.47	0.18
Mean Cell Vol. (MCV) (fL)	85.2±7.0	82.5±2.9	87.9±9.1	0.20	0.12	0.36
MCH (pg)	29.2±2.8	28.3±1.2	30.1±3.8	0.29	0.24	0.50
MCHC (g/dL)	34.2±0.7	34.3±0.7	34.2±0.8	0.82	0.83	0.90
RDW	13.8±1.1%	14.3±0.9%	13.3±1.2%	0.14	0.07	0.30
Platelet Count ($x10^3$)	217.5±42.7	202.5±45.2	232.5±37.8	0.24	0.28	0.92
White Blood Cell (WBC) ($x10^3$)	6.9±2.8	6.1±2.1	7.6±3.4	0.37	0.42	0.77
Neutrophils	56.6±11.1%	54.8±6.2%	58.4±14.9%	0.60	0.55	0.53
Lymphocytes	32.0±9.7%	33.5±7.4%	30.4±12.1%	0.61	0.50	0.34
Monocytes	8.6±3.5%	8.2±2.9%	8.9±4.2%	0.73	0.71	0.63
Eosinophils	3.3±4.2%	5.1±5.5%	1.5±1.1%	0.14	0.15	0.76
Basophils	0.6±0.2%	0.6±0.2%	0.5±0.2%	0.37	0.52	0.15
Neutrophil # ($x10^3$)	4.1±2.7	3.4±1.2	4.9±3.6	0.35	0.39	0.89
Lymphocytes # ($x10^3$)	2.0±0.6	2.0±0.8	2.1±0.6	0.93	0.87	0.19
Monocytes # ($x10^3$)	0.6±0.3	0.5±0.4	0.6±0.2	0.58	0.52	0.53
Eosinophils # ($x10^3$)	0.13±0.1	0.1±0.1	0.1±0.1	0.80	0.74	0.37
Basophils # ($x10^3$)	0.01±0.03	0.02±0.02	0	0.34	0.36	1.00
Reticulocytes	1.4±0.6%	1.5±0.5%	1.3±0.6%	0.70	0.74	0.48

Values are mean±SD. Between analysis for SCT vs. NORM; Within participants analysis accounts for individual's variability with matched pairs. Bold values are considered significant. Pair within is the Wald p-value for the random variability.

Table 6. Camp Blood Chemistries

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
Glucose (mg/dL)	89.9±17.9	81.8±8.5	98.0±21.9	0.12	0.09	0.44
Sodium (mmol/dL)	138.2±1.5	138.0±1.5	138.3±1.5	0.71	0.66	0.47
Chloride (mmol/dL)	104.5±0.9	104.2±0.4	104.8±0.4	0.30	0.43	0.22
Potassium (mmol/dL)	4.2±0.2	4.1±0.2	4.3±0.2	0.11	0.17	0.66
Carbon Dioxide (mmol/dL)	24.4±1.7	24.4±1.7	24.4±1.8	0.97	0.97	0.99
Uric Acid (mg/dL)	6.5±1.3	7.2±1.3	5.8±0.7	**0.05**	**0.02**	0.25
Total Protein (g/dL)	7.3±0.4	7.2±0.4	7.4±0.3	0.50	0.46	0.63
Calcium (mg/dL)	9.3±0.3	9.2±0.4	9.4±0.1	0.36	0.34	0.69
Phosphorus (mg/dL)	4.4±0.5	4.4±0.5	4.4±0.6	0.96	0.95	0.46
Total Bilirubin (mg/dL)	0.9±0.4	0.9±0.4	0.9±0.4	0.84	0.56	0.14
CPK (IU/L)	1081.9±531.1	1347.6±477.2	816.3±472.6	0.08	0.17	0.34
LDH (IU/L)	216.8±45.7	218.8±60.3	214.8±30.8	0.89	0.90	0.66
AST (IU/L)	39.8±12.6	44.4±12.3	35.1±12.0	0.21	0.35	0.21
ALT (IU/L)	34.2±11.6	34.7±12.7	33.7±11.6	0.89	0.92	0.25
ALK Phosphatase (IU/L)	84.3±32.2	90.6±35.9	78.0±29.9	0.52	0.57	0.73
GGT (IU/L)	21.3±12.7	24.5±14.4	18.2±11.1	0.41	0.43	0.99
Amylase (U/L)	106.3±53.1	130.7±62.5	82.0±29.5	0.12	0.23	0.23
Iron (µg/dL)	66.8±24.7	65.7±19.5	67.8±30.9	0.89	0.75	0.16
Cholesterol (mg/dL)	163.7±24.7	168.5±26.8	158.8±23.7	0.52	0.53	0.90
Triglycerides (mg/dL)	87.8±54.6	100.8±72.0	74.7±31.0	0.43	0.39	0.61
HDL Cholesterol (mg/dL)	54.2±12.8	48.8±13.2	59.5±10.9	0.15	0.10	0.39
LDL Cholesterol (mg/dL)	92.0±27.6	99.6±27.5	84.4±27.9	0.36	0.34	0.68

Values are mean±SD. Between analysis for SCT vs. NORM; Within participants analysis accounts for individual's variability with matched pairs. Bold values are considered significant. Pair within is the Wald p-value for the random variability. *insufficient blood samples from multiple positionn-matched participants.

As shown in table 4, Hb-A (between: P<0.0001; within: P<0.0001), Hb-A2 (between: P=0.0002; within: P=0.004), and Hb-S (between: P<0.0001; within: P<0.0001) were the only significant hemoglobin measures following camp. Camp activities produced no significant differences in any CBC measures for the participants or groups, as shown in Table 5. Table 6 shows the only blood chemistry measure, uric acid (SCT=7.2±1.3mg/DL vs CON=5.8±0.7mg/DL; between P=0.05, within P=0.02), that showed significance, following camp activities being increased compared to CON.

Post-Season

Table 7. Postseason Hemoglobin Levels

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
Total Hb**	14.6±0.5	14.6±0.5	14.6±0.6	0.94	0.96	0.39
Hb-A**	75.9±20.3	56.7±0.6	95.1±0.5	**<0.0001**	**<0.0001**	0.95
Hb-A2**	3.2±0.4	3.6±0.2	2.9±0.2	**0.004**	**0.0003**	0.91
Hb-S**	19.2±20.2	38.3±0.7	-	**<0.0001**	**<0.0001**	>0.99
Hb-F**	0.5±0.3	0.4±0.2	0.5±0.3	0.60	0.49	0.28
Hb-C**	-	-	-	-	-	-

Values are mean±SD. Between analysis for SCT vs. NORM; Within participants analysis accounts for individual's variability with matched pairs. Bold values are considered significant. Pair within is the Wald p-value for the random variability. **missing data from 1 position-matched with SCT (n=5 CON; n=5 SCT).

Table 8. Postseason Complete Blood Counts

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
Red Blood Cells (RBC) (x10^6)**	5.0±0.4	5.2±0.2	4.7±0.4	0.07	**0.04**	0.95
Hematocrit (Hct)**	43.3±1.9%	43.4±1.5%	43.1±2.3%	0.82	0.81	0.97
Mean Cell Vol. (MCV) (fL)**	87.6±5.6	83.6±3.9	91.5±4.0	**0.004**	**0.01**	0.25

(Table Cont'd.)

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
MCH (pg)**	29.6±2.3	28.0±1.5	31.1±1.9	**0.02**	**0.02**	0.39
MCHC (g/dL)**	33.8±0.6	33.6±0.3	33.9±0.7	0.44	0.31	0.30
RDW**	13.5±1.0%	14.2±0.5%	12.8±0.8%	**0.01**	**0.01**	0.36
Platelet Count ($x10^3$)**	240.7±65.0	206.2±50.8	275.2±62.8	0.17	0.09	0.55
White Blood Cell (WBC) ($x10^3$)**	6.4±2.1	5.5±1.0	7.2±2.7	0.36	0.24	0.36
Neutrophils**	47.6±13.5%	50.3±13.8%	45.0±14.4%	0.50	0.57	0.51
Lymphocytes**	38.3±11.1%	34.2±7.0%	42.4±13.6%	0.16	0.27	0.35
Monocytes**	9.5±3.0%	9.5±2.6%	9.6±3.6%	0.96	0.97	0.42
Eosinophils**	3.7±4.9%	4.9±6.9%	2.5±1.3%	0.43	0.46	0.69
Basophils**	0.9±0.4%	1.0±0.5%	0.8±0.2%	0.44	0.38	0.70
Neutrophil # ($x10^3$)**	3.0±1.2	2.8±1.0	3.2±1.5	0.73	0.65	0.31
Lymphocytes # ($x10^3$)**	2.5±1.5	1.9±0.4	3.1±1.9	0.18	0.19	0.66
Monocytes # ($x10^3$)**	0.6±0.2	0.5±0.1	0.6±0.2	0.25	0.34	0.42
Eosinophils # ($x10^3$)**	0.2±0.2	0.2±0.3	0.2±0.1	0.59	0.68	0.39
Basophils # ($x10^3$)**	0.1±0.1	0.1±0.1	0.1±0.1	>0.99	>0.99	0.27
Reticulocytes**	1.3±0.5%	1.4±0.5%	1.2±0.6%	0.71	0.69	0.85

Values are mean±SD. Between analysis for SCT vs. NORM; Within participants analysis accounts for individual's variability with matched pairs. Bold values are considered significant. Pair within is the Wald p-value for the random variability. **missing data from 1 position-matched with SCT (n=5 CON; n=5 SCT).

Table 9. Postseason Blood Chemistries

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
Glucose (mg/dL)**	98.6±27.6	90.8±15.4	106.4±36.3	0.49	0.40	0.52
Sodium (mmol/dL)**	137.4±1.3	136.8±1.3	138.0±1.0	0.11	0.14	0.49
Chloride (mmol/dL)**	103.2±1.4	103.1±1.6	103.3±1.3	0.87	0.86	0.89
Potassium (mmol/dL)**	4.2±0.5	4.4±0.7	4.1±0.3	0.47	0.44	0.99
Carbon Dioxide (mmol/dL)**	21.4±3.9	22.4±1.6	20.4±5.4	0.52	0.45	0.61
Uric Acid (mg/dL)**	6.3±0.8	6.8±1.0	5.8±0.2	**0.05**	**0.04**	0.68

(Table Cont'd.)

	All	SCT	NORM	Between P-value	Within P-value	Pair Within
Calcium (mg/dL)**	9.5±0.3	9.4±0.3	9.5±0.2	0.55	0.50	0.68
CPK (IU/L)**	506.9±287.4	567.3±334.4	446.5±254.7	0.32	0.54	0.26
LDH (IU/L)**	216.7±70.1	213.2±83.8	220.1±63.2	0.91	0.89	0.50
AST (IU/L)**	37.3±11.0	35.6±8.6	39.0±13.8	0.60	0.65	0.53
ALT (IU/L)**	32.0±14.1	28.0±12.7	36.0±15.7	0.41	0.40	0.86
ALK Phosphatase (IU/L)**	84.3±28.9	84.6±34.2	84.0±26.7	0.98	0.98	0.80
GGT (IU/L)**	19.4±14.7	17.0±3.6	21.8±21.5	0.59	0.64	0.58
Iron (µg/dL)**	97.8±29.4	110.6±24.8	85.0±30.4	**0.05**	0.18	0.23
Cholesterol (mg/dL)**	174.7±23.9	183.6±27.1	165.8±18.7	0.18	0.26	0.41
HDL Cholesterol (mg/dL)**	59.4±14.2	56.0±15.3	62.9±13.9	0.50	0.48	0.97

Values are mean±SD. Between analysis for SCT vs. NORM; Within participants analysis accounts for individual's variability with matched pairs. Bold values are considered significant. Pair within is the Wald p-value for the random variability. *Insufficient blood samples from multiple position-matched participants. **missing data from 1 position-matched with SCT (n=5 CON; n=5 SCT).

Table 7 displays the Hb-A (between: P<0.0001; within P<0.0001), Hb-A2 (between: P=0.004; within P=0.0003) and Hb-S (between: P<0.0001; within: P<0.0001) significance found, following the season. The CBC significant measures following the season were in red blood count (5.2±0.2x10^6 vs 4.7±0.4x10^6; within P=0.04), mean cell volume (MCV) (83.6±3.9FL vs 91.5±4.0FL; between P=0.004, within P=0.01), mean cell hemoglobin (MCH) (28.0±1.5PG vs 31.1±1.9PG; between P=0.02, within P=0.02), and red cell distribution width (RDW) (14.2±0.5 vs 12.8±0.8; between P=0.01, within P=0.01), as shown in Table 8. Of the blood chemistries from postseason samples, uric acid (SCT=6.8±1.0mg/DL vs CON=5.8±0.2mg/DL; between P=0.05. within P=0.04) and iron (SCT=110±24.8ug/DL vs CON 85.0±30.4ug/DL; P=0.05), were the only two to display significant increases compared to CON, as shown in table 9.

Time Course Data

Camp

All CBC measures across time showed no significance (P>0.11 for all). No significance was shown for any blood chemistry measures, as well (P>0.08).

Season

The significance found across time for the season were the adjusted changes for sodium (-0.44, -1.22 to 0.35 vs. 1.13, 0.38 to 1.87; P<0.006) and GGT (0.95, -2.73 to 4.62 vs. 5.32, 1.77 to 8.88; P<0.02) measures. All CBC measures showed no significance.

CHAPTER 4. DISCUSSION

The purpose of the study was to explore the biological, physiological, and psychological effects of division I-A collegiate level training on individuals with SCT. We hypothesized minimal differences in ability to perform, and some hemorheological changes would be more noticeable in the SCT athletes post-camp and post-season. Preseason blood draws exhibited the most significance across all three blood panels whereby major alterations in hemoglobin, complete blood count and blood chemistries were observed. As expected, hemoglobin panels confirmed the SCT status of the players over all three time-points. In addition, some of the WBCs, neutrophils, lymphocytes, etc. measures averages were slightly elevated in SCT participants, at baseline, as expected. The participants coming back from being off from mandatory sport administered activities, led us to assume those baseline differences would be seen. In spite of tremendously great weather conditions for the majority of our study, post-camp CBC values produced no significant differences.

The lack of significant differences was shocking, because previous researcher's findings of significant differences in different blood measures, such as leukocyte activation and platelet count during or following exercise. [9, 23] The lack of significant difference could be due to the well-trained staff or athletes and their awareness or abilities, to know when breaks are necessary. The lack of significance could also be due to the low severity of the environment during the entire study. Could hotter or colder temperatures have been more of a factor in causing complications due to SCT? Following all season activities, a RBC significance was found when participants were looked at as being the same. The RBC significance is possibly brought about from the strenuous season and needs of the body during season. For these highly trained athletes, during season, stroke volumes were probably elevated, for practices and competitions, to

increase cardiac outputs, ultimately trying to provide the body with more oxygenated cells to be delivered through the body. In addition to the RBC significance, iron was found to be significantly higher in SCT at postseason as well. On each protein of the hemoglobin, iron is capable of carrying oxygen, which explains the elevated values found between the two measures, RBC and iron. In relations to the RBC, red cell distribution width (RDW), mean cell volume (MCV) and mean cell hemoglobin (MCH) all showed to be significant as well, most likely due to the significant increase of RBC. RDW showed that in the RBC, the sizes of the erythrocytes varied greatly. Those cell size differences could be due to the SCT blood disorder and how the cells life spans are shorter or sickled, from hypoxia. With shorter cell life span in SCT erythrocytes, the question is could the varying sizes of RBC be significantly greater because the reduced time to produce more cells in attempts to maintain a certain RBC level? Or, could the varying sizes be accounting for the sickled shape cells? Highly correlated to the significance found in the RBC, MCV too showed postseason significance differences, with CON being higher. With increased RBC in the blood, the volume of the number of cells also should increase, but the inverse occurred. With the MCV increased in the CON could the sickled cells be the reason we see lower values in the SCT participants. Lastly, the MCH significance is also correlated to the significance found in iron and RBC. Though as previously mentioned, if the cells are sickled there is less amounts of oxygen able to be carried, which could justify the significantly lower MCH in SCT

During the preseason, levels of amylase showed significance but did not appear significant at any other time point. The preseason significance in amylase, could be due to possible carbohydrate loading by the players in attempts to have a great amount of energy for the first day of activities. A measure that showed significance during all three time-points was uric

acid, which has been said to be associated with oxidative stress and shown increased levels following exercise. The association to oxidative stress has said to be due to prevention of the kidney to clear out uric acid with lactate, that is produce during exertion. [24] The explanation for significant values found in our study after camp could be attributed to the kidneys inability to filter the blood. With relatively high levels of creatine phosphokinase (CPK) in SCT participants, following camp, the blood osmolality could have been high as well adding more to be filtered. In addition to CPK breakdown, if the athletes are hypohydrated, blood osmolality can be affected negatively leading to high levels of uric acid. An unlikely possibility for the significant uric acid values found post-camp and postseason, can stem from a study that found elevated uric acid levels, post-exercise, as a sign of hypertension and poor health. [25] With the strains of performing football related task, hypertension can occur, the pressure of blood against the arterial walls will continually fluctuate. So, the question is whether elevated levels of uric acid are an indicator of poor health or a protective antioxidant for oxidative stress, which can be investigated further.

Across time there were no major effects on blood count markers, which proved that there was no interaction between the SCT and CON groups. The only significant measures found were of blood chemistry measures, sodium and GGT. The sodium difference could be due to the need for SCT participants need to stay hydrated, so rather than storing fluids with from high levels of salt ingested, they were just drinking larger amounts of water. Inversely, the large difference could due to higher sodium intake by the CON participants, like beverages or meals with more sodium. The significance found in GGT, a marker of liver function, metabolic syndrome, and oxidative stress, could be due to the increased levels of iron and RBC, which has been said to be an influencer on elevated GGT levels, when the RBC are damaged. [26] In addition, GGT could

have been affected by participants consumption of fluids before and/or during activities. Could the significance reduce to be a non-significant difference, later than the 24 hours post blood draw?

Strengths and Weaknesses

Strengths of this study was the precision of the position-matched participants. The pairs helped us to not only compare CON and SCT participants, but to get as close as possible to the same height, weights and amounts of work done by each pair. In addition to being close pair matches, the variety of positioned participants, helped us to see various task performed, and their effects. All twelve participants were highly trained, all year round, which could ultimately be the reason for the lack of significance found. Despite a lack of significance, the study took place in a sport setting rather than a laboratory. The setting helped us understand additional environmental or task specific activities that may influence participants' hemorheology. The participants overall showed great compliance and adherence to the design. The compliance of the athletes also came with outstanding support from the athletic training staff and team members in helping with day-to-day, when needed. Some of the study's weaknesses include a small sample size, and the insufficient data which removed multiple position-matched groups. In the most scientific manner, there was an overall lack of "events" that occurred throughout study which restricted our ability to determine markers in the blood that may induce an "event". In this study, post-camp and postseason blood draws occurred at least 24 hours after the last practice or game, thereby limiting our understanding of the acute effects of the activities on hemorheology. Last, the environmental stress was lower than typical for this year across all athletes and time-points, posing a relative weakness to evaluate the environmental effects in SCT.

Conclusion

As expected, SCT did show significant differences during the various time-points, but many questions and continued research would be able to help identify other influences, such as the environment, not being highly trained, etc. that cause complications due to SCT. The study only showed significance across time in sodium and GGT, providing us information that these highly trained participants, SCT and CON, show individual differences in blood measures but their differences are parallel to their position matched partners.

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APPENDIX. IRB APPROVAL FORMS

CONSENT TO PARTICIPATE IN A RESEARCH STUDY
INFORMED CONSENT

Title of Study: Sickle cell trait in collegiate athletes: effects on mental, physical, and emotional resiliency.

We give you this consent form so that you may read about the purpose, risks and benefits of this research study.

- The main goal of a research studies is to gain knowledge that may help future athletic trainers and coaches make better decisions about appropriate treatment for athletes with the sickle cell trait.
- You have the right to refuse to take part, or agree to take part now and change your mind later on.
- Please review this consent form carefully and ask any questions before you make a decision.
- Your participation is voluntary and will not influence your team standing or your practice training.
- By signing this consent form, you agree to participate in the study as it is described.

1- Investigators:

The following investigator will be available for questions about this study:

Principal Investigator: Neil M. Johannsen, Ph.D.
Phone: 225-578-5314
Email:njohan1@lsu.edu

Co-Investigators: Jack Marucci
Phone: 225-578-2451
Email: jmarucc@lsu.edu
Shelly Mullenix
Phone: 225-578-8642
Email: smulle1@lsu.edu
Timothy S. Church, M.D., M.P.H., Ph.D.
Email: tim.church@pbrc.edu
Brian Harrell, M.D.
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Email: gsspielmann@lsu.edu
Brian Irving, Ph.D.

Email: brianirving@lsu.edu
Jennifer Rood, Ph.D.
Email: Jennifer.rood@pbrc.edu

Tiffany Stewart, Ph.D.
Email: tiffany.stewart@pbrc.edu
Ray Castle, Ph.D., ATC, LAT
Email: rcastl1@lsu.edu
LeKeitha Poole, Ph.D.
Email: lpoole2@lsu.edu

Neil Johannsen, Ph.D. directs this study. We expect about 30 athletes will be enrolled in this study per year; ~15 with sickle cell trait and ~15 position matched controls. The study will take place over a period of about 1 year for each athlete and may include athletes from multiple sports.

2- Performance Site:

This study will take place at Louisiana State University-Baton Rouge Campus.

3- Purpose of the Study:

This study is designed to explore the biological, physiological, and psychological effects of elite level training on individuals with the sickle cell trait. In addition, we aim to determine mediating factors affect your ability to perform in your sport including hydration status, body temperature, and cardiovascular responses.

4- Participant Inclusion:

Participants will include athletes form Louisiana State University who are active in their sport during the season. Athletes with confirmed sickle cell trait will be included in this study as determined by the health history provided by the LSU medical staff or trainers. Other athletes matched for sport position (ex. linebacker) will be serve as the study control group.

You are eligible for this study if you are:

- An athlete at Louisiana State University
- Capable and willing to give written informed consent, understand exclusion criteria
- Cleared by a Louisiana State University physician to participate in your given sport.

You are **NOT eligible** for this study if you have any conditions the Louisiana State University physician regards as being too risky to participate in your sport. Additionally, you can be excluded for any other medical, psychiatric or behavioral factors that in the judgment of the Principle Investigator may interfere with study participation or the ability to follow the protocol.
The investigators of this study will have access to the data collected under the supervision of your physician during your exam to determine final study inclusion.

5-Study Procedures:

The study for which you are volunteering could last an entire competitive season (1 year). The study will include 4 time points for data collection:

1. Pre-Seaseon
2. Pre-Competition Season
3. Post-Competition Season
4. Before, During, and/or After Practice

Summary of Visit Schedule

Procedure	Pre-season	Pre-competition	Post-competition	Practice
Informed Consent	**X**			
Screening	**X**			
Blood Samples	**X**	**X**	**X**	**X**
Body Weight	**X**			**X**
Physiological Monitoring	**X**	**X**	**X**	**X**
Urine Samples				**X**
Sweat Samples				**X**
Fluid Intake				**X**
Body Temperatures				**X**
Psychological Data	**X**	**X**	**X**	**X**

Screening Visit

At this visit, or during multiple visits, you will undergo your standard tests as part of being an athlete in your sport. This visit may or may not occur before you were offered to participate in the study. Study personnel will examine and record some of the information collected during your physical exam and pre-practice testing. The following information and procedures will be used as part of this study:

- Height and weight
- Medical history and medication use
- Blood pressure and heart rate – by a cuff placed around your arm and pulse
- Body composition

If you choose not to participate in this study, none of your data collected will be used by the study investigators for this research.

Study Visits

You will have assessments taken Pre-season, Pre-competition, and Post-Competition. These assessments will include physiological and psychological monitoring and blood. Pre-season data collected will serve as the baseline measurements. The follow-up measurements will examine the chronic changes associated with practice and competitive seasons throughout the year.

- Psychological Data - Sleep quality questionnaire, sources/symptoms of psychological well-being, and activation state
- Blood Collection - a small amount of blood will be taken from an arm vein to look for known markers of sickle cell changes related to training and stress. About 20 mL of blood (2 Tablespoons) will be drawn at each of the 3 visits.
- Physiological Monitoring - A monitor will be worn around your chest. The monitor can assess heart rate and the variability in your heart rate, a measure of heart health.

Practice – before, during, and/or after

In addition to the periodic study visits listed above, you will be monitored before, during, and after practice. **The practice will be conducted under the direction of coaches.** Study investigators will have no influence on the physical activity training and the Louisiana State University Athletic Department medical staff will be present throughout the practices. However, we will monitor and collect observational data. Practice assessments will include body weight, physiological monitoring, fluid intake, body temperatures, sweat collection, and urine collection.

- Body weight
- Physiological Monitoring - A monitor will be worn around your chest. The monitor can assess physical activity level, heart rate, breathing rate, and GPS data (distance and speed running).
- Fluid intake – the amount and type of fluid you ingest prior to and during practice may be recorded to use in together with body weight to estimate sweat rate.
- Body temperature – skin temperature will be taken by an infrared thermometer, core temperature will be assessed by an ingestible pill.
- Sweat patch application and removal – An adhesive patch with an absorbent cotton material is placed on the body and remains during practice. The absorbent material will be weighed before application and after removal to estimate sweat rate. The sweat will be analyzed for.
- Urine sample – a small sample of urine will be provided and analyzed for color, specific gravity, osmolality, and sodium, potassium, and chloride.

In addition to these measures, other data collected by the Louisiana State University Athletic Department medical staff or team physicians may be used as data in this study. All additional measurements will be performed if the Louisiana State University Athletic

Department medical staff or study physicians **deem it is medically necessary**. These measurements could include urine and blood chemistries, psychological data, body temperature, hard outcome data (for example - incidence of heat-related illness, muscle cramps, injuries, concussion), and other data collected during practice from existing practices by the LSU teams that may be related to the sickle cell trait. In addition, in these cases, urine and/or blood samples may be used to determine the use of performance enhancing supplements. If you are volunteering as a control participant, the additional measurements will also be conducted if medical "event" occurs in your pair matched sickle cell trait participant.

- Blood Collection - a small amount of blood will be taken from an arm vein to look for known markers of sickle cell changes related to training and stress. About 20 mL of blood (2 Tablespoons) will be drawn.
- Urine Collection - Urine sample – a small sample of urine will be provided and analyzed for color, specific gravity, osmolality, and sodium, potassium, and chloride.
- Psychological Data - Sleep quality questionnaire, sources/symptoms of psychological well-being, and activation state
- Outcome data – hard outcomes, such as muscle cramping, dehydration and IV infusion, hyper- or hypothermia, injuries, concussion, and others, may be collected as part of the study.
- Other data – data collected during practice from existing measures may be used as part of this study. This data will be related to the ability to assess or predict future heat-related illness or muscle cramping.

6- Risks/Discomforts:

Sweat Collection: There are no risks associated with this test. There is a small possibility there may be some redness or irritation if you happen to be allergic to the adhesive on the collection patches.

Core temperature: There are no risks associated with this test. The pills provided to the participants will be unused and sterile prior to use. The pills contain metal; participants who ingest the pills should not undergo an MRI scan until the pill has passed in the feces (~24-48 hours). The LSU Athletic Department medical staff and team physicians will be notified who has ingested the pills in order to avoid the risk of MRI. In the event that an injury occurs that requires transport to another facility for evaluation using an MRI, a note will be sent with the participant informing the staff to use an alternative method, other than an MRI, for diagnostic purposes.

Heart rate monitor: There are no known risks associated with the monitor.

Blood draws: Trained and medically certified personnel will and draw your blood. The training for the personnel may include physician monitored and/or phlebotomy certifications. There may be minimal discomfort and bruising and/or bleeding where the needle is inserted for finger prick blood sampling. There is a possibility of pain, bruising,

and/or infection at the site of the needle insertion. Aseptic (sterile) technique and trained personnel minimize this risk.

Loss of Confidentiality: Completing questionnaires may result in a breach in confidentiality of personal data. Participants will be assigned ID numbers and information that could identify a participant will not appear in publications. However, the data collected as part of this observational study can be shared with your sport-specific coaches and athletic trainers.

Unknown risks: In addition to the risk listed above, you may experience a previously unknown risk or side effect.

7- Benefits:

We cannot promise any benefits from your being in the study. However, possible benefits include:

- Information about your general health
- Information regarding your body's response to exercise in an outdoor environment

8-Alternatives to Participation:

There are no alternatives to the study described in this consent. You have the choice at any time not to participate in this research study. If you choose not to participate, any benefits to which you are entitled will not be affected in any way.

9- Injury/Illness or Questions:

If you have any questions about your rights as a research volunteer, you should call Dennis K. Landin, Ed.D., Institutional Review Board Office at 225-578-8692. If you have any questions about the research study, contact Neil Johannsen, Ph.D. at 225-578-5314 during regular working hours. If you believe you have a research-related injury or medical illness, contact Louisiana State University Athletic Department medical staff or team physician.

10-Privacy:

Every effort will be made to maintain the confidentiality of your study records. Results of the study may be published; however, we will keep your name and other identifying information private. Other than as set forth above, your identity will remain confidential unless disclosure is required by law.

11-Early Study Withdrawal:

Neil Johannsen, Ph.D. can withdraw you from the study for any reason or for no reason. You may withdraw from the study at any time without penalty. Possible reasons for withdrawal include injury, the presence of an old or existing injury that may be deemed risky, sufficient medical history deemed too risky for testing.

12-Additional Information:

During the course of this study there may be new findings from this or other research that may affect your willingness to continue participation. Information concerning any such new findings will be provided to you.

13-Charges for Participation:

None

14-Payments for Participation:

You will be compensated for your time and the procedures involved with this study. Specifically, you will be compensated $25 for each blood draw/laboratory visit for a total of $75 if you complete the study. In addition, if you are required to have additional blood drawn (separate from normal athletic or medical training purposes) due to a medical event during practice, you will be compensated $25 for each event. The monies will be provided in check form soon after each visit or event.

15- Compensation for study-related injury or medical illness:

No form of compensation for medical treatment or for other damages (i.e., lost wages, time lost from work, etc.) is available from Louisiana State University. In the event of injury or medical illness resulting from the research procedures in which you participate, you will be referred to a treatment facility. Medical treatment may be provided at your own expense or at the expense of your health care insurer (e.g., Medicare, Medicaid, Blue Cross-Blue Shield, Dental Insurer, etc.), which may or may not provide coverage.

16- Signatures:

The study has been discussed with me and all my questions have been answered. I understand that additional questions regarding the study should be directed to the study investigators. I agree with the terms above and acknowledge that I have been given a copy of the consent form.

_____

Printed Name of Volunteer

_____ _____

Signature of Volunteer Date

_____

Date of Birth of Volunteer

_____ _____

Signature of Person Administering Informed Consent Date

Neil M. Johannsen, Ph.D.
Principal Investigator

17- Tissue/Specimen Storage for Future Research or Use

Biospecimens for future research:
You are being asked to allow some of your blood to be stored and used for research at a later time. These bodily materials are called biospecimens. The donation of biospecimens in this study is optional. No matter what you decide to do, it will not affect your study participation. You will still be allowed to take part in the study even if you don't want your specimens to be collected and used for future research. Some biospecimen samples will be stored and used for the study and other biospecimen samples will be stored for future studies. The collection of samples may give scientists valuable research material that can help them to develop new diagnostic tests, new treatments, and new ways to prevent diseases. If you agree to have your samples stored, you can change your mind up until the end of the study.

The samples will be stored indefinitely. If you agree to donate your samples, they may be given to other investigators for future research as well. The future research may take place at Louisiana State University and may involve Louisiana State University Researchers in this study. The future research may not take place at Louisiana State University and may not be reviewed by Louisiana State University's Institutional Review Board. For privacy and confidentiality, your biospecimens will be labeled with a unique series of letters and numbers. Louisiana State University will store your biospecimens with this unique identifier and the minimum number of personal identifiers to meet laboratory standards. The research done with your specimens may help to develop new products in the future, or may be used to establish a cell line or test that could be patented or licensed. You will not receive any financial compensation for any patents, inventions or licenses developed from this research.

Making your choice about future research:
Please read about each biospecimen below. It is your choice which samples will be collected, stored and used for future research for this study or future studies. After reading about each biospecimen below, sign your name next to "Yes" or "No" to show your choice about the collections for this research study and for future research studies.

Blood
If you give permission, approximately 1 tablespoon of blood will be stored by this study. Your stored samples may be tested at Louisiana State University or other locations used in future research.
Do you give permission for your blood to be collected and used in future research by this study?

Yes, I give permission _____

SIGNATURE

No, I do not give permission _____

SIGNATURE

Urine

If you give permission, approximately 1 tablespoon of urine will be stored by this study. Your stored samples may be tested at Louisiana State University or other locations used in future research.

Do you give permission for your blood to be collected and used in future research by this study?

Yes, I give permission _____

SIGNATURE

No, I do not give permission _____

SIGNATURE

If you decide you would like to withdraw your consent to use your samples, you will be able to do so until the study ends. After the study ends, you will not be able to withdraw your consent to use your samples because investigators will not know which one is yours. You must provide a written request to have your samples destroyed.

For destruction of your samples, you can contact the Principal Investigator at:

Neil Johannsen, PhD
Louisiana State University
112 Huey P. Long Fieldhouse
Baton Rouge, LA 70803

VITA

Michael E. Owens was born in Carrollton, Georgia, in 1992, but was raised in Houston Texas. He has always been interested in sports, but more importantly wanting to be involved in them, due to his desire stay active and compete. After participating in college football, during his first few years of undergraduate studies, he knew helping others, by maximizing and coaching sports performance, was what he really wanted to accomplish. Following his break from obtaining his bachelors in 2015, he ultimately decided to return to school for his masters, in search of greater knowledge, and opportunities to get closer to his goals.